University of Alberta

A Mechanism for Self-Pruning in Lodgepole Pine

by

Clark G. Protz

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

Department of Renewable Resources

Edmonton, Alberta

Fall 1999

National Library of Canada
Acquisitions and Bibliographic Services
395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque nationale du Canada
Acquisitions et services bibliographiques
395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence

Our file Notre référence

The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de cette thèse sous la forme de microfiche/film, de reproduction sur papier ou sur format électronique.

The author retains ownership of the copyright in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

L'auteur conserve la propriété du droit d'auteur qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

0-612-47083-0

Canada

University of Alberta

Library Release Form

Name of Author: Clark Graham Protz

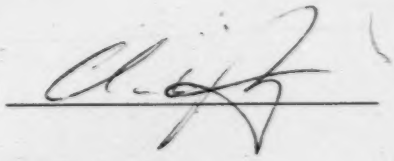
Title of Thesis: A Mechanism for Self-Pruning in Lodgepole Pine.

Degree: Master of Science

Year this Degree Granted: 1999

Permission is hereby granted to the University of Alberta Library to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly, or scientific research purposes only.

The author reserves all other publication and other rights in association with the copyright in the thesis, and except as hereinbefore provided, neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form whatsoever without the author's prior written permission.



June 8th 1999

2165 Collingwood Street,
Vancouver
British Columbia
V6R 3K8

University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled A Mechanism for Self-Pruning in Lodgepole Pine submitted by Clark Graham Protz in partial fulfillment of the requirements for the degree of Master of Science.

JUNE 8TH 1999

Dr V. J. Lieffers

Dr U. Silins

Dr R. Knowles

Dr J. J. Zwiazek

Abstract

I examined the role of branch hydraulic architecture in the process of self-pruning in dense *Pinus contorta* (var Latifolia) stands. I tested the hypothesis that sapwood produced in lower crown branches under shaded growth conditions has anatomical characteristics which confer greater hydraulic resistance to water flow. The sapwood of lower crown branches had smaller tracheid lumen diameters and lower hydraulic permeability than upper branch sapwood. Artificial drought stress was imposed on upper and lower crown branches by de-activating fractions of the active sapwood. Lower crown branches experienced greater foliage mortality after one growing season, indicating they were pre-disposed to drought stress. Transpiration and stomatal conductance were lower in lower crown branches, indicating greater stomatal regulation of transpirational water loss. This new evidence suggests that self-pruning does not occur solely due to low light, but that the underlying mechanism involves the hydraulic architecture of the lower crown branches.

Acknowledgments

Over the course of a graduate degree there are many people who provide essential support and encouragement as well as keeping the student focused on attaining excellence.

I would like to express my sincere appreciation of the wise council, guidance and patience of my supervisors Vic Lieffers and Uldis Silins. I would also like to thank my friends and colleagues Ken Stadt, Simon Landhäusser and Annie DesRochers for their technical assistance and countless thought provoking discussions which have vastly enriched my graduate experience. The conscientious field assistance of Sarah Lieffers, Vashti Thompson, and Justine Karst - particularly their willingness to spend many days counting pine needles - is very much appreciated.

For the tremendous encouragement and compassion of my friend and fiancé Erin Fukushima, as well as her considerable editorial contribution and demand for excellence, my heartfelt thanks.

Most of all I am forever grateful to my parents Sheila and Steve Protz for their unwavering support and belief in me through my years of undergraduate university at Exeter, England, and graduate university here in Canada.

This research was jointly funded by Weyerhaeuser Canada Ltd., Sunpine Forest Products Ltd., Canadian Forest Products Ltd., Millar Western Industries Ltd., Weldwood of Canada Ltd. and the National Science and Engineering Research Council, with an additional grant from the Circumpolar Boreal Institute.

Table of Contents

		Page
1.0	**Chapter 1**	
	A literature review on crown dynamics and crown recession	1
1.1	Light limitation hypothesis	3
1.2	Branch water stress hypothesis	6
1.3	Summary of the branch water stress hypothesis	11
1.4	Specific hypotheses	12
1.5	Literature cited	13
2.0	**Chapter 2**	
	The role of branch hydraulic architecture in crown recession	17
2.1	Introduction	18
2.2	Materials and Methods	21
	2.21 Site description	21
	2.22 A. Foliage mortality and physiological responses to imposed drought stress	21
	2.23 B. Branch xylem hydraulic permeability and anatomy	25
2.3	Results	29
	2.31 A. Foliage mortality and physiological responses to imposed drought stress	29
	2.32 A. Foliage mortality and physiological responses to imposed drought stress	31
2.4	Discussion	39
2.5	Literature cited	44
3.0	**Chapter 3**	
3.1	Synthesis	47
3.2	Literature cited	51
4.0	**Chapter 4**	
4.1	Appendices: Statistical analysis summary tables	52

List of Tables

Page

Table 1.1. Results of ANOVA's of (a) hydraulic conductivity reduction, and (b) sapwood area disruption in the imposed drought stress experiment. Upper and lower branches (position) were disrupted by high and low disruption treatments (treatment). Values indicate P>f.

53

Table 1.2. Results of ANOVA's of foliage mortality due to imposed drought stress in upper and lower branches (position) of closed canopy trees. Values indicate P>f.

53

Table 1.3. ANOVA results for measurements of physiological responses to xylem disruption in upper and lower branches (position) of open and closed canopy trees (density). P>f values for stomatal conductance (g_s), transpiration rates (E) and branch water potential (Ψ) are shown.

54

Table 1.4. Results of ANOVA's of hydraulic permeability per unit leaf area (k_L), hydraulic permeability per unit sapwood cross-sectional area (k_s) and Huber values (HV) in upper and lower control branches in closed and open canopy trees. Values indicate P>f.

54

Table 1.5. ANOVA results of tracheid diameter, length, and bordered pit frequency in earlywood xylem of upper and lower crown branches (position) of (a) closed and open canopy trees (density), (b) and (c) separately within each density. Values indicate P>f.

54

Table 1.6. ANOVA results of a chronological sequence of annual growth ring measurements in lower crown branches from closed and open canopy trees (density) from the last 10 years. The first 5 years (10-6) and the last 5 years (5-1) were analyzed separately. P>f values for the ratio of earlywood width to total ring width for each year, and total growth ring width are shown.

55

List of Figures

Page

Figure 1. Degree of sapwood disruption in the imposed drought stress experiment, in upper and lower branches (n=18 trees). (a) Conducting sapwood area disruption (% of control + SE). (b) Hydraulic conductivity K_h (ms^{-1} $x10^{-4}$ + SE); low disruption treatments reduced k_h by 45% and high disruption by 72% ($p = 0.011$).

33

Figure 2. Foliage mortality (% + SE) from low and high sapwood (s/w) disruption treatments, phloem disruption treatments, and controls in upper and lower branches of closed canopy trees (n = 20). Phloem disruption caused greater mortality than controls ($p = 0.0012$) but less than sapwood disruption ($p = 0.029$). Foliage mortality differed between high and low sapwood disruption treatments ($p = 0.005$), and between crown positions ($p < 0.001$).

34

Figure 3. Physiological responses (+ SE) to xylem disruption in upper and lower branches of open and closed canopy trees (n = 20). Stomatal conductance (g_S) and transpiration rates showed identical trends and only g_S (mmol m^{-2} s^{-1}) is shown. Branch water potential (Ψ, MPa) is plotted with corresponding g_S for control and sapwood disrupted branches in (a) closed canopy and (b) open canopy trees. Ψ did not differ significantly with crown position, treatment or tree canopy type. Stomatal conductance differed with crown position ($p < 0.001$) and treatment ($p < 0.001$).

35

Figure 4. (a) Hydaulic permeability per unit leaf area k_L (m^2 $x10^{-5}$ + SE), (b) Hydraulic permeability per unit sapwood cross-sectional area k_S (m^2 + SE), and (c) Huber values (HV, m^2 m^{-2} + SE) of upper and lower control branches in closed and open canopy trees (n = 24). Both k_L and k_S differed between crown positions ($p = 0.033$ and 0.052 respectively) and tree canopy types ($p = 0.062$ and 0.039 respectively).

36

Figure 5. Earlywood xylem tracheid anatomy in upper and lower crown branches of closed and open canopy trees. (a) Tracheid diameters (μm + SE) differed between crown positions ($p = 0.0341$, n = 21 trees), but not between stand densities ($p = 0.376$). (b) Tracheid length (μm + SE, n = 20 trees) showed little variation between crown positions and stand densities ($p = 0.437$ and 0.840 respectively). (c) The frequency of bordered pits (per 100 μm^{-1} of tracheid length + SE, n = 20 trees) differed little between upper and lower branches in closed canopy trees ($p = 0.261$) but the frequency was higher in lower branches of open canopy trees ($p < 0.001$).

37

Figure 6. Chronological sequence of annual growth ring measurements in lower crown branches from closed and open canopy trees from the last 10 years (branches were 11 years old). Year 10 corresponds to 1987 growth and year 2 to 1997 growth. (a) Proportion of each years' growth ring width laid down as earlywood (+ SE, n = 20 trees). The earlywood proportion in both canopy densities was similar over the first 5 years ($p = 0.148$) but closed canopy proportions were marginally less in the last 5 years ($p = 0.063$). (b) Total annual growth ring width including both early and latewood (+SE, n = 20 trees). Growth rings were similar overall in both crown densities in the first 5 years ($p = 0.087$) and in the last 5 years ($p = 0.640$).

List of Plates

Plate 1. Branch water stress hypothesis.

11

Plate 2. Cross section of a lower branch from a closed canopy tree. (A) Untreated section showing the non-conducting heartwood (centre black) and conducting sapwood (white). (B) The high disruption treated section of the same branch showing the dye stained actively conducting sapwood and non-conducting radial pattern caused by drilling. Plastic pegs were inserted through drill holes to prevent bulk water flow or healing growth.

23

Chapter 1.

A literature review on crown dynamics and crown recession.

Many conifer species of the boreal forest exhibit the phenomenon of self pruning or crown recession (Putz, *et al.* 1984). This is the natural die-back of the lower branches of tree crowns as they grow taller, and is particularly noticeable in stands that have regenerated after a disturbance such as fire or clear-cutting. These stands are typically even-aged and comprise only one or two species. These stands are often extremely dense, and therefore reach the crown closure stage of stand development relatively soon. This high density of trees with crowns touching those of neighbouring trees causes intense intra-specific competition for light with the result that height growth far exceeds lateral crown growth (Maguire & Hann, 1990). Consequently, live crowns in many conifer species including lodgepole pine (*Pinus contorta* var. *latifolia*, Lamb), jack pine (*Pinus banksiana*, Lamb) and loblolly pine (*Pinus taeda* L.) are short and narrow (Baldwin & Peterson, 1997). This introduction provides a brief review of the literature and discusses potential mechanisms responsible for crown recession.

Crown recession is unlikely to be caused by a purely genetic mechanism, given evidence of environmental influences such as stand density and site quality (Takahashi 1996; Baldwin & Peterson 1997). One theory is central to a number of hypothetical mechanisms behind crown recession. The 'carbon balance model' (Mäkelä 1995) suggests that each branch within a crown is self sufficient in terms of carbon produced by photosynthesis in leaves on that branch, and the respiratory cost of its supporting sapwood and heartwood tissues. A branch will grow and stay alive only as long as it represents a net benefit to the tree (in terms of carbon gain). Conversely it will die when it becomes a carbon burden. After crown closure, trees allocate photosynthates to height growth rather than foliage growth elsewhere in the crown (i.e. horizontal crown growth). This notion has much support, and forms the central core of both the classic hypothesis describing crown recession which is based upon light limitation, and also a new hypothesis posed in this study involving branch water relations. According to the classic hypothesis light is a limiting resource to lower branches (Sampson & Smith 1993, Waring *et al.* 1981), causing productivity of leaves to decline below the point at which the tree can support the branch, thus, causing death (Schultz &

Matthews 1993). A new 'branch water stress' hypothesis is presented and tested in this study. This new hypothesis describes how the changes in the hydraulic architecture of the tree as a whole, the lowest branches in particular, may lead to branch death. The lower branches may be unable to meet the transpirational demand of their leaves, resulting in early stomatal closure and therefore reduced photosynthesis leading to death.

These two hypotheses both suggest plausible mechanisms behind the carbon balance explanation for self pruning. If light is limiting to lower branches due to an increasing foliage mass in the upper crown, and photosynthesis is reduced, then as the branch ages it will have difficulty meeting the carbon requirements for respiration of its own tissues. If branch water supply becomes limiting, this will also represent a photosynthetic limitation. In order to avoid xylem embolism damage (cavitation of xylem water columns) due to very low xylem water potentials, stomata are likely to be closed earlier in the day to regulate transpirational demand, subsequently limiting photosynthesis.

Before exploring this new branch water stress hypothesis in detail, it is pertinent to consider the traditional light limitation hypothesis which has long been accepted by many forest ecologists.

The Light limitation hypothesis.

The penetration of light to lower levels of a tree crown depends on stand characteristics such as crown density, as well as solar angle (Smith, Sampson & Long 1991, Mencuccini & Grace 1996b). For instance, open grown lodgepole pines usually develop full crowns (high ratio of live crown length to total tree height) because incident light to all branches is similar in quantity (mols photons m^{-2}) and spectral composition. However, differences in light exist between the foliage at the ends of the branches and that nearer to the centre of the crown because of self-shading (Waring, Newmann & Bell 1981). In a dense fire regenerated stand, lodgepole pine will tend to have a very low live crown ratio. As much as two thirds of the lower crown will not receive maximum light since the penetration of

photosynthetically active radiation through the canopy declines curvilinearly from the top to the bottom of the crown (Vose & Swank 1990).

Maguire & Hann (1990) found that with Douglas fir the higher the stand density the more rapid the rate of crown recession. Codominant trees experienced more rapid crown recession than dominant or suppressed trees. They argued that dominant trees have low crown recession rates because they are expanding or maintaining their occupation of aerial growing space. Codominants have to compete more intensely for growing space and therefore vertical crown growth takes a high priority over the maintenance of lower whorls. Suppressed or overtopped trees have very slow height growth, since the space above them has already been occupied. They have to wait for a disturbance to create conditions (i.e., a gap in the canopy) in which they can compete. Maguire & Hann (1990) argued that suppressed trees must struggle to maintain as large a live crown as possible in order to maximize light interception, and therefore will have a slow rate of crown recession. Maguire & Hann's work does not fully support the light limitation hypothesis. Suppressed trees growing in a very low light environment below the canopy should be experiencing the most rapid crown recession. Douglas Fir, however, is more shade tolerant than pine, which may explain their observations.

Sampson & Smith (1993) reported that the order of importance of canopy characteristics on light penetration were; leaf area index (LAI), which is a measure of leaf cover (m^2 leaf area / m^2 ground area); the degree of aggregation of foliage or 'clumpiness'; average leaf angle; and lastly, vertical distribution of foliage (i.e., depth and shape of crown). This implies that crown depth (and therefore crown recession) has the least effect on light penetration through the crown. Long & Smith (1989) found that the greatest stemwood growth efficiency in lodgepole pine stands for a given leaf area index (LAI) occurs where live crown ratio is low and the crown depth is restricted by high stem density. The lowest growth efficiency per unit growing space was found in widely spaced stands with deep crowns. Deep crowns typically have only a slightly larger leaf area than short crowns, but it is distributed as a thin 'shell' surrounding an inner core largely devoid of foliage (Baldwin & Peterson

1997). Traditionally this crown form was thought to represent the most effective capture of light, and thus was associated with the most efficient growth. However, this inner core is really a 'dead volume' within the tree. It has a very low ratio of leaf weight to branch weight, which corresponds with a relatively poor carbon balance for the crown as a whole.

The development of a foliage free inner core corresponds with increasing age and is associated with a decline in mean annual increment (MAI) and leaf area index (LAI). The development of the inner core occurs after stand closure and coincides with a decline in stemwood production. Long and Smith (1992) make the distinction between vigorous and efficient stands. Trees with long crowns and high absolute growth rates are vigorous, but inefficient with respect to the occupied ground space. Conversely, efficient trees may have unimpressive individual growth rates, yet higher stand level volume growth. The critical issue is the efficiency of the whorls at the crown base. In trees with full crowns, lower whorls have large branches. These big branches have a low leaf weight per unit branch weight, and thus the poorest carbon balance within the crown. The branches have to grow longer in order for their foliage to intercept light, and so the branch diameter at the base must increase, corresponding to the necessary increase in mechanical bracing (Cannell, Dewer 1991). As the tree ages until crown closure of the stand, lower branches require a larger mass of supportive tissue for a constant photosynthetic area. At crown closure these bottom branches become heavily shaded by surrounding trees and productivity of their foliage declines. At this point, the branch cannot export carbon, and likely cannot even meet its own respiratory needs. Branch mortality is imminent because the branch is a carbon drain on the tree ('carbon-negative'). However, the exact mechanism causing death is uncertain. It is possible that carbon is not allocated to such branches by the rest of the crown as growth will be preferentially directed to the leader and upper branches. The 'carbon-negative' branch will then decline and die but may remain attached to the tree for some time.

The branch water stress hypothesis.

A brief overview of hydraulic architecture theory.

Discussion of a new hypothesis for crown recession involving branch water stress demands a brief consideration of the historical work on tree hydraulic architecture. A pioneering model was developed in 1964 by Shinozaki *et al.*, called the pipe model theory, which relates total leaf area to sapwood area of the stem of trees by considering each unit of leaf area as being supplied by a constant area of xylem. This is analogous to a pipe, or given number of pipes, delivering water to each leaf. This work has been supported by numerous modellers who estimated tree leaf area from the cross-sectional area of the stem at breast height (Waring, Schroader & Owen 1982; Whitehead, Edwards & Jarvis 1984; Valentine 1985; Keane & Wheetman 1987). However, this model is complicated by the fact that the functional state of xylem varies with age and can be altered by environmental stresses, such as water deficit (Berninger & Nikinmaa 1994). Much work has followed in developing more accurate models for predicting leaf area. Long & Smith (1987) realized that the sapwood component of stem cross sectional area varies with height from breast height to the base of the live crown, which lead to the development of a non-linear model unbiased by stand density and site effects. Coyea and Margolis (1992) found that the leaf area / sapwood area ratio for balsam fir was correlated with both site quality and tree age. They devised an improved prediction of leaf area by regressing 5-year basal area and crown length with sapwood area. Their findings that crown length has an influence on sapwood area has significance to the branch water stress hypothesis.

Within sapwood, the ease with which the individual 'pipes' or elements conduct water to the foliage can be measured as hydraulic permeability according to the Poisielle-Hagan Law for ideal capillaries, as modified by Tyree & Ewers (1991) in equation 1;

$$k_h = (\pi\rho/128\eta) \sum (d^4_i) \qquad \text{-(equation 1.)}$$

where k_h = hydraulic permeability (m s^{-1}), ρ = density of the fluid (kg m^{-3}), η =

dynamic viscosity of the fluid (MPa s^{-1}), d = pipe diameter (m). Confusion exists in the literature between the terms hydraulic conductivity (a property of both the perfusate and the porous media), hydraulic resistance (the reciprocal of conductivity) and hydraulic permeability (solely a property of the porous media, as the viscosity of the perfusate is already accounted for). Many workers mistakenly refer to hydraulic conductivity (K), rather than permeability, however this study will refer to permeability.

The hydraulic permeability of sapwood in tree stems and branches determines part of the hydraulic resistance of the water flow pathway through the soil-plant-atmosphere continuum. Water flow through sapwood increases proportionally with the fourth power of the vessel (or tracheid) lumen diameters, because the cell walls cause resistance to water flow (Mark & Crews 1973). In conifers, the main point of resistance within the tracheid is not friction along the cell wall, however, but where water flows through the membrane at the inter-pit pore (Mark & Crews 1973). The aperture of the pit pore increases with tracheid maturity because the membranes become more fragile and are easily damaged. Sperry, Perry & Sullivan (1991) found older pit pores in vessels of *Populus tremuloides* had appertures of up to 0.5 μm whilst young ones were typically only 0.08μm. Thus, pit pores confer less resistance to flow as they age, but tracheids also become more vulnerable to cavitation as they age.

Under conditions of drought stress, the water potential in the xylem is reduced. This reduction can be sufficient to cause the water column to break in an event known as cavitation. The cavitation process involves the dissolved gases in the water coming out of solution and forming bubbles within the tracheid. An air filled tracheid is referred to as an embolism, and forms a blockage to water movement in that xylem element. There have been several attempts to explain how embolisms form, including radiation, mechanical shock, and rapid pressure changes. The theory with the greatest support is the 'air-seeding' hypothesis as described by Sperry & Tyree (1988b). The air-seeding mechanism starts with the diffusion of air into the tracheid from an adjacent air space through the pit pores. Once the pressure

difference across the pore meniscus exceeds the sap surface tension holding the meniscus in place, the air and water vapour can spread through the pore to adjacent tracheids. As the water potential gradient between the soil and the leaves increases during drought, embolisms act as nuclei for further cavitations and embolisms tend to 'spread' more readily. This has been described as a destructive and potentially fatal chain reaction, or 'runaway catastrophic xylem dysfunction' (Sperry & Tyree 1988). The tree has some measure of control in the form of regulating stomatal aperture, and hence transpirational demand (Jones & Sutherland 1991). Closing stomata during the day to reduce water demand has the major drawback of reducing photosynthesis, and many species are thought to operate very close to the brink of catastrophic xylem failure to minimize this loss of productivity (Tyree & Sperry 1988).

Vulnerability to cavitation is also influenced by tracheid dimensions. Large lumen diameters and pit pore apertures are more vulnerable to cavitation because the water meniscus across the aperture has a lower surface tension, therefore air will come out of solution and spread to neighbouring tracheids more readily at higher water potentials (Sperry, Perry & Sullivan 1991). Using equation 1 Sellin (1990) calculated that up to 95% of sapwood water flow occurs in the largest diameter class of tracheids. If we can estimate the proportion of tracheids within this size class, it is possible to predict the vulnerability to cavitation of sapwood from different parts of the tree. Although larger diameter tracheids have the advantage of higher hydraulic permeability, in boreal winters they can be a liability as they are much more prone to cavitation caused by freeze-thaw cycles (Sperry & Sullivan 1992). In addition to tracheid lumen diameter, tracheid length is also important in determining hydraulic permeability. Longer tracheids have higher hydraulic permeability because water has to pass through fewer pits per unit length of the pathway (Pothier *et al.* 1989b).

There is one major problem with the proposed branch water stress mechanism for crown recession related to the role of embolisms in reducing transpirational water supply to foliage; it has been shown that embolisms are readily reversible (Edwards *et al.* 1994). In angiosperms, slightly higher xylem water potentials in the early spring due to 'root pressure' have been found to be sufficient to 'flush' the sapwood of most

embolisms formed during the previous year. Such positive xylem water potentials are believed not to occur in gymnosperms, but embolisms can be reversed even at potentials slightly below zero (Sperry & Tyree 1988). The reversal process is thought to occur when the water potential across the pit membrane exceeds the partial pressures of the air / water vapour mixture in the embolised conduit. The gases then redissolve in the surrounding functional conduits and the embolism is dispersed (Edwards *et. al* 1994). If embolisms in branch sapwood are reversible, then their role in reducing permeability may not be so significant in the long term. Further, the formation of emboli may not play a major role in sapwood hydraulic permeability of lower branches, since the effect of lower water potentials may be canceled by the reduced vulnerability of the tracheids due to their small lumens. If poor permeability is due to the greater resistance of the small tracheid lumens and infrequent bordered pits, then embolisms are likely to be of less importance than tracheid diameter, length, and pit frequency in the question of branch water stress causing crown recession.

Xylem hydraulic permeability varies according to several other factors, including tree age and species, tracheid maturity, whether it was formed in earlywood or latewood, and the location of the tracheid in the tree (Sperry, Alder & Easlack 1993; Sellin 1994; Mencuccini & Grace 1996). For example, the different stages of the hydraulic pathway from soil to stomata each represent different proportions of the overall hydraulic resistance within a tree (where hydraulic resistance can essentially be taken as inversely proportional to hydraulic permeability). Sellin (1993) reports that for Norway spruce the lowest hydraulic resistance per unit cross sectional area of sapwood is found in the stem, whilst the primary branches and the roots have the highest hydraulic resistance. However, Yang & Tyree (1994) report that the largest contribution to whole tree hydraulic resistance for *Acer spp.* comes from the leaves and petioles. From these reports we might draw the analogy of the mammalian vascular system. Vessels become smaller in diameter towards peripheral areas (from arteries to capillaries), conferring greater resistance to flow.

Differences in hydraulic permeability of sapwood within conifer crowns could be the principle mechanism for preferentially supplying water to different parts of the

crown during drought periods. Differential hydraulic permeability in sapwood is the result of differences in tracheid anatomy, therefore it should be possible to detect these anatomical traits in the sapwood of branches in the lower crown. For instance, Sperry & Tyree (1990) found that latewood tracheids (and vessels in angiosperms) were less vulnerable to embolisms than earlywood tracheids due to their smaller lumen diameters, but also had lower hydraulic permeability as a result. Branches at the crown base in Scots pine have narrower growth rings, a lower proportion of total sapwood area, and a higher proportion of low hydraulic permeability latewood (Mencuccini & Grace 1996). This leads to an overall reduction in whole branch water conductance at the bottom of the canopy in Scots pine. There is some evidence that anatomical sapwood differences exist in some species, and that they may be caused by different light conditions experienced by upper and lower branches during sapwood development. Sellin (1993) found that *Picea abies* showed differences in hydraulic resistance between upper and lower crown positions. Lower branches had significantly higher resistance, and all branches had much higher resistance in a tree grown in shade conditions.

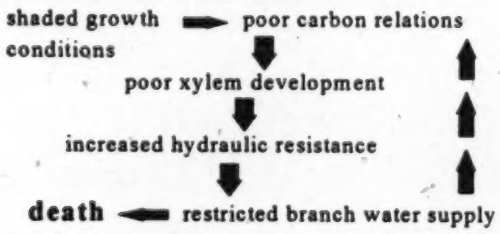
A possible mechanism for the difference in anatomy due to light conditions is that in many conifers the largest percentage of water conductance in the branch sapwood is through the two most recent growth rings (Pothier *et al.* 1989). Sapwood retains water conducting properties for many years, however there is a declining permeability gradient moving inwards from the cambium to the sapwood - heartwood boundary. This is primarily the result of embolisms and 'plugging' of the tracheid pits in the innermost rings which occurs as part of the conversion to heartwood (Sellin 1990). Light limitation may act by restricting the resources available for growth to the branch, resulting in very small annual growth rings. The majority of the conductive capacity of the branch is therefore restricted to the relatively small cross-sectional area of recently formed sapwood. Therefore branch productivity will be limited by both poor light and water availability, and future growth rings may have even smaller tracheid diameters. In addition to annual growth effects on lumen diameter, slow growth rates may also reduce the frequency of pits

per tracheid. This has been found to be the largest determinant of resistance to water flow (Mark & Crews 1973) and is therefore potentially a more important contributor to hydraulic resistance than lumen diameters.

A summary of the branch water stress hypothesis.

Anatomical differences may exist between tracheids of branches from the lower crown and the tree top resulting in the lower branches having inferior water conducting properties. These anatomical differences may be a result of lower branches growing in shaded conditions. During drought stress, stomatal closure may regulate evaporative water loss to maintain branch water potentials and minimize the formation of embolisms. In this case, productivity of the branch would be limited because low hydraulic permeability means water supply to the foliage cannot meet transpirational demand, therefore limiting photosynthesis. Over successive seasons of low productivity, the carbon balance of the branch would become increasingly negative leading to death and eventual abscission. Plate 1 illustrates the chain of events described by the hypothesis:

Plate 1. Branch water stress hypothesis



To date there have been no studies in pine exploring differences in the xylem properties known to influence hydraulic permeability between sapwood produced in shade conditions in lower crown branches and upper branches grown in full light. It is unknown whether branch hydraulic permeability decreases in lower crown positions, or whether foliage of lower branches prone to self-pruning suffers a greater degree of water stress than upper crown foliage. Little is known of the carbon fluxes

between these branches and the rest of the tree, and whether they are supported as carbon sinks no longer able to contribute to tree growth.

Specific hypotheses

In this study I exaggerated branch water stress by disrupting sapwood equally in upper and lower crown branches, and measured foliage mortality and physiological responses with the following specific hypotheses;

(a) Exaggerated water stress leads to greater foliage mortality in lower crown branches over the duration of a growing season.

(b) Foliar transpiration rates and stomatal conductance are reduced in lower crown branches compared to upper crown branches, even when measured in similar light conditions, but xylem water potentials are uniform throughout the crown.

Additional work measured hydraulic permeability and anatomical parameters of tracheids to test the following specific hypotheses;

(c) Hydraulic permeability (expressed per unit cross-sectional area of active sapwood and per unit leaf area supplied) is higher in upper crown branches than in lower crown branches.

(d) Sapwood anatomical properties (tracheid diameter, length, and bordered pit frequency) allow for more efficient water transport in upper crown branches than in lower crown branches.

LITERATURE CITED

Berninger, F., and Nikinmaa, E. 1994. Foliage area - sapwood relationships of Scots pine (*Pinus sylvestris*) trees in different climates. Can. J. For. Res. **24**: 2263-2268.

Cannell, M.G.R., and Dewar, R.C. 1991. Carbon allocation in trees: a review of concepts for modeling. Advances in Ecological Research **25**: 60-102.

Baldwin, C.V., and Peterson, K.D. 1997. Predicting crown shape of loblolly pine trees. Can. J. For. Res., **27**: 102-107.

Coyea, M.R., and Margolis, H.A., 1992. Factors affecting the relationship between sapwood area and leaf area of balsam fir. Can. J. For. Res. **22**: 1684-1693.

Edwards, W.R.N., Jarvis, P.G., Grace, J. and Moncrief, J.B. 1994. Reversing cavitation in tracheids of *Pinus sylvestris* L. under negative water potentials. Plant, Cell and Environment, **17**: 389-397.

Jones, H.G., and Sutherland, R.A. 1991. Stomatal control of xylem embolism. Plant, Cell and Environment, **14**: 607-612.

Keane, M.G., and Weetman, G.F. 1987. Leaf area-sapwood cross-sectional area relationships in repressed stands of lodgepole pine. Can. J. For Res. **17** :205-209.

Long, J.N, and Smith, F.W., 1987. Leaf area - sapwood relations of lodgepole pine as influenced by stand density and site index. Can. J. For. Res. **18**:247-250.

Long, J.N, and Smith, F.W., 1989. The influence of canopy architecture on stemwood production and growth efficiency of *Pinus contorta* var. *Latifolia*. Journal of Applied Ecology, **26**: 681-691.

Long, J.N., and Smith, F.W. 1992. Volume increment in *Pinus contorta* var. *latifolia*: the influence of stand development and crown dynamics. Forest Ecology and Management, **53**: 53-64.

Maguire, D.A., and Hann, D.W. 1990. Constructing models for direct prediction of 5-year crown recession in southwestern Oregon Douglas-fir. Can. J. For. Res. **20**:1044-1052.

Mäkelä, A. 1995. A carbon balance model of growth and self-pruning in trees based

on structural relationships. Forest Science, 43(1): 7-24.

Mark, W.R., and Crews, D.L. 1973. Heat-pulse velocity and bordered pit condition in living Engelmann spruce and Lodgepole pine trees. Forest Science, 4:291-296.

Mencuccini, M., and Grace, J. 1996. Developmental patterns of above-ground hydraulic conductance in a Scots pine (*Pinus sylvestris*) age sequence. Plant, Cell and Environment, **19**: 939-948.

Mencuccini, M., and Grace, J. 1996b. Hydraulic conductance, light interception and needle nutrient concentration in Scots pine stands and their relations with net primary productivity. Tree Physiology, **16**: 459-468.

Pothier, D., Margolis, H.A., and Waring, J. 1989. Patterns of change of saturated sapwood permeability and sapwood conductance with stand development. Can. J. For. Res. **19**: 432-439.

Pothier, D., Margolis, H.A., Poliquin, J., and Waring, R. 1989. Relation between the permeability and the anatomy of jack pine sapwood with stand development. Can. J. For. Res. **19**: 1564-1570.

Putz, F.E., Parker, G.C., and Archibald, R.M., 1984. Mechanical abrasion and intercrown spacing. Am. Midl. Nat., **112**: 24-28.

Sampson, D.A., and Smith, F.W. 1993. Influence on canopy architecture on light penetration in lodgepole pine (*Pinus contorta* var. *Latifolia*) forests. Agricultural and Forest Meteorology, **64**:63-79.

Schultz, H.R., and Matthews, M.A., 1993. Xylem development and hydraulic conductance in sun and shade shoots of grapevine (*Vitis vinifera* L.): evidence that low light uncouples water transport capacity from leaf area. Planta, **190**:393-406.

Sellin, A. 1990. Main factors affecting hydraulic conductance of the xylem of Norway spruce. Fiziologiya Rastenii, **37:3** 450-456.

Sellin, A. 1991. Hydraulic conductivity of xylem depending on water saturation level in Norway spruce (*Picea abies* (L.) Karst.). Plant Physiol., **138**: 466-469.

Sellin, A. 1993. Resistance to water flow in xylem of *Picea abies* (L.) Karst. trees

grown under contrasting light conditions. Trees, **7**: 220-226.

Sellin, A. 1994. Sapwood-heartwood proportion related to tree diameter, age, and growth rate in *Picea abies*. Can. J. For. Res. **24**: 1022-1028.

Shinozaki, K., Yoda, K., Hozumi, K., and Kira, T. 1964a. A quantitative analysis of plant form - the pipe model theory. 1 Basic analyses. Jpn. J. Ecol. **14**:97-105.

Shinozaki, K., Yoda, K., Hozumi, K., and Kira, T. 1964a. A quantitative analysis of plant form - the pipe model theory. 2. Further evidence of the theory and its application in forest ecology. Jpn. J. Ecol. **14**:133-137.

Smith, F.W., Sampson, D.A., and Long, J.N. 1991. Comparison of leaf area index estimates from tree allometrics and measured light interception. Forest Science, **37**: 1682-1688.

Sperry, J.S., Alder, N.N., and Eastlack, S.E. 1993. The effect of reduced hydraulic conductance on stomatal conductance and xylem cavitation. Journal of Experimental Botany, **44**: 1075-1082.

Sperry, J.S., Perry, A.H., and Sullivan, J.E.M. 1991. Pit Membrane Degradation and Air-Embolism Formation in Ageing Xylem Vessels of *Populus Tremuloides* Michx. .Journal of Experimental Botany, **42**: 1399-1406.

Sperry, J.S., and Sullivan, J.E.M., 1992. Xylem embolism in response to freeze-thaw cycles and water stress in ring-porous, diffuse-porous, and conifer species. Plant Physiol. **100**: 605-613.

Sperry, J.S., and Tyree, M.T. 1988. Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress ? Answers from a model. Plant physiol., **88**: 0574-0580.

Sperry, J.S., and Tyree, M.T. 1988b. Mechanism of water stress-induced xylem embolism. Plant Physiol. **88**: 581-587.

Sperry, J.S., and Tyree, M.T., 1990. Water-stress-induced xylem embolism in three species of conifers. Plant, cell and Environment. **13**: 427-436.

Takahashi, K. 1996. Plastic response of crown architecture to crowding in understory trees of two co-dominating conifers. Annals of Botany **77**: 159-164.

Tyree, M.T., and Ewers, F.W. 1991. Tansley Review No. 34. The hydraulic

architecture of trees and other woody plants. New Phytol., **19**: 345-360.

Valentine, H. 1985. Tree growth models: deviations employing the pipe-model theory. J. Theor. Biol. **117**: 579-585.

Waring, R.H., Newmann, K., and Bell, J. 1981. Efficiency of tree crowns and stemwood production at different canopy leaf densities. Forestry, **54**:129-137.

Waring, R.H., Schroader, P.E., and Owen, R. 1982. Application of the pipe model theory to predict canopy leaf area. Can. J. For. Res. **12**: 556-560.

Whitehead, D., Edwards, W.R.N., and Jarvis, P.G. 1984. Conducting sapwood area, foliage area, and permeability in mature trees of *Picea sitchensis* and *Pinus contorta*. Can. J. For. Res. **14**: 940-947.

Yang, S., and Tyree, M.T. 1994. Hydraulic architecture of *Acer saccharum and A. rubrum*: comparison of branches to whole trees and the contribution of leaves to hydraulic resistance. Journal of Experimental Botany, **45**: 179-186.

Chapter 2

The role of branch hydraulic architecture in crown recession.

INTRODUCTION

Lodgepole pine (*Pinus contorta* var. latifolia, Lamb) regenerates naturally after fire or harvesting with site-preparation, often forming dense even-age stands. These stands reach crown closure relatively soon thereafter, causing intra-specific competition for light within crowns (Maguire & Hann 1990). At this stage of stand development crown recession begins. Crown recession is thought to be a result of suppressed growth of branches at the crown base due to low light conditions (Waring *et al.* 1981; Sampson & Smith 1993). Low light at the bottom of crowns may not provide sufficient energy to support leaves and maintain a positive carbon balance within the branch.

This study explores a new explanation for crown recession based on branch water stress. Suppressed growth caused by low light availability may adversely affect the quality of the new xylem produced in the annual growth ring of lower branches. This, in turn, impairs the water supply to the foliage causing water stress and stomatal closure. The background for this new hypothesis is based on more general research conducted on tree hydraulic architecture and properties of tree stems. When water supply to leaves cannot meet evaporative demand, more frequent stomatal closure occurs (Jones & Sutherland 1991), which limits the photosynthetic capacity of the foliage. Eventually, the branch cannot export carbon to the rest of the tree and therefore becomes a carbon drain (Schultz & Matthews 1993). As growth of new cells is the lowest priority use of available carbon (Waring & Pitman 1985), when carbon is limited one might speculate that phloem and xylem production would be reduced in quantity and fluid transport efficiency. As a result, phloem capacity may be insufficient to import sufficient carbon to meet respiratory requirements. If respiratory requirements exceed carbon production and import, new growth will cease and existing cells will die (Ryan & Yoder 1997). This hypothetical process has not previously been reported.

This new branch water stress hypothesis for the mechanism causing crown recession assumes that lower light levels at the crown base provide poor growing conditions for the formation of new sapwood to maintain water supply to the foliage.

Sperry & Tyree (1990) found trunk xylem of conifers grown in shade (understory) had smaller annual growth increments and greater proportions of latewood, which is of little value in conducting water. Further, hydraulic permeability is a function of the anatomy of the tracheids, in particular tracheid lumen diameter, length, and frequency of bordered pits (Pothier *et al.* 1989b). The hydraulic permeability of the tracheid is theoretically proportional to the fourth power of the lumen diameter (Tyree & Ewers 1991), thus highly permeable sapwood has a high proportion of large cells. The length of the tracheid and the frequency of pits also affect the flow rate since the inter-cell connection at the pits is the point of highest hydraulic resistance in the flow pathway along multiple tracheids (Zimmermann 1978). The longer the tracheids and the more pits per unit length, the greater the hydraulic permeability of the xylem.

Much work has been reported in the literature on general hydraulic architecture in relation to stems and roots, but few people have studied branches. In one such study of *Acer spp.* Yang and Tyree (1994) reported 35% of whole tree hydraulic resistance was from crown xylem, with only 15% from trunk xylem and the rest from foliage. Other authors reached similar conclusions (Edwards *et al.* 1982; Tyree 1988; Zimmermann 1983). The foregoing research suggests branch xylem plays an important role in whole-tree water relations, however there are no reported studies of branch hydraulic architecture in the context of a potential mechanism for branch mortality.

In the present study I examined the intermediate steps in the branch death process. Specifically, whether shaded lower branches exist on the brink of water starvation, and whether the physiological response to this starvation is to close stomata, shutting down transpiration and photosynthesis. To determine if heavily shaded lower branches are on the brink of water starvation, further drought stress was artificially imposed upon them by disrupting sapwood. The responses to this treatment were measured by recording foliage mortality on each branch over one growing season. I hypothesized that the mortality of foliage on lower branches would be greater than that of the upper branches. To determine physiological responses to this imposed water stress, xylem water potentials, transpiration rates,

and stomatal conductance were measured. I hypothesized that foliage of branches in lower crown positions would have lower transpiration rates and stomatal conductance due to stomatal response to increased hydraulic resistance, but would maintain similar xylem potentials throughout the crown due to stomatal control as found by Sperry & Pockman (1993).

I measured the sapwood permeability per unit leaf area supported (k_L) of branches in the upper and lower parts of the crown to determine if there was a difference among branches growing in different light regimes. I hypothesized that k_L of upper branches would be greater than in the shaded lower branches, and that upper branches would therefore represent more efficient pathways for water flow. I hypothesized that any differences in k_L would be related to differences in tracheid anatomy. Thus I measured tracheid properties of branches from the upper and lower crown positions to test the hypothesis that the tracheids formed under low light conditions in the lower branches had smaller diameters, and were shorter with fewer pits per unit length. Recent growth in lower branches should have a lower earlywood / latewood ratio as low light and inadequate water supply limit the development of earlywood during the summer (Sperry & Tyree 1990).

A natural aging process of the branches could also explain different growth rates of branches in different crown positions in older trees, as described in the maturation hypothesis of height growth limitations in old trees (Ryan & Yoder 1997). The maturation hypothesis describes a slowing of the growth of meristematic tissue as the number of cell divisions increases over time. If branches decline solely as a result of age, regardless of light conditions and water supply, this process must be uncoupled from the postulated hydraulic effects. To uncouple effects due to branch age from effects of hydraulic limitations, physiological, hydraulic and anatomical measurements were compared between closed canopy trees and same-aged open canopy trees with full crowns.

MATERIALS AND METHODS

Site description

Two sites were selected for sampling and measurements for the project. Both sites were pure even-aged lodgepole pine stands twenty years old and 4-5 m tall, regenerated naturally by drag scarification after harvesting. The two sites were approximately two kilometers apart on a west facing slope in the Rocky Mountains upper foothills region (53°20'N; 117°30'W). To quantify stand densities and diameter at breast height (DBH), trees were sampled within ten 3x3 m randomly distributed plots in each stand. Site one had a mean DBH of 4.02 cm and density of 7444 stems / ha compared to 8.17 cm and 4556 stems / ha for site 2. These densities were sufficiently high for the trees to be exhibiting crown recession, but still have branch diameters large enough for experimental treatments. Both stands also had areas of low density trees with almost full live crowns. Site one had an elevation of 1420 m, while site two was at 1485m. Both sites were classified as the tall bilberry / Arnica Lodgepole pine ecosite phase of the upper foothills natural region (Natural Resources Canada, 1996).

A. Foliage mortality and physiological responses to imposed drought stress.

Ten densely grown trees on each site were randomly chosen and marked in early May 1998 for the imposed drought stress (drilling) experiment, with an additional ten for the supporting physiological measurements. All trees exhibited considerable mortality of live crown (crown length to tree height ratio was 40-50%). Within each tree one upper and one lower whorl, each with at least five live branches, were selected for treatment application. The lower whorl was the one above the last live whorl at the crown base. The designated upper whorl was three years old and still in full light conditions.

Foliage mortality was assessed by counting the total number of live fascicles on each branch before treatment in May 1998 and again in September. For every branch within each whorl, all of the live fascicles (defined as having more than one green needle) along the main leader from bole to emergent bud were counted. Each

year of growth was counted separately using bud scars to identify each year's growth.

The aim of the xylem disruption treatments was to uniformly reduce the cross-sectional area of conducting sapwood to restrict water supply and therefore simulate drought conditions. Two target levels of sapwood disruption were used for comparison - 40% and 80%. Water flow to all parts of the branch was reduced by drilling small holes radially through the branch - like the spokes of a wheel. The cross - sectional area of sapwood disrupted by a hole from a 2mm drill bit was calculated to determine the number of holes required to disrupt sapwood by the target levels in a branch of known diameter. These calculations also accounted for the greater proportion of sapwood in upper branches which lacked heartwood.

The 40% and 80% treatments were applied to two different branches within each whorl. Each hole was immediately plugged with 2mm diameter plastic wire to prevent healing and water movement across the hole.

The exact level of disruption caused by the treatments was calibrated using 30cm long internodes of upper and lower branches from separate trees (n = 30). Each internode was then divided into three 10 cm sections and these were randomly assigned to control, 40% or 80% treatments. Adjacent sections were assumed to share the same hydraulic properties and comparable flow rates. The hydraulic conductivity measurement apparatus (after Booker 1984) generated a tension head of 7.5 kPa. The sampling procedure and hydraulic conductivity measurement technique for the calibration is discussed in detail with the hydraulic permeability work in section B below. Treated branch segments were drilled to the same specifications as the field treatments and their hydraulic conductivity and dye-flow patterns were recorded. Plate 2 shows the dye-stained sapwood cross-sectional area for a branch section treated for high disruption and its associated undisrupted control. Hydraulic conductivity (K_h) is a property of both the fluidity of the permeate (water) and the permeability of the porous medium (sapwood), and was calculated from the following equation;

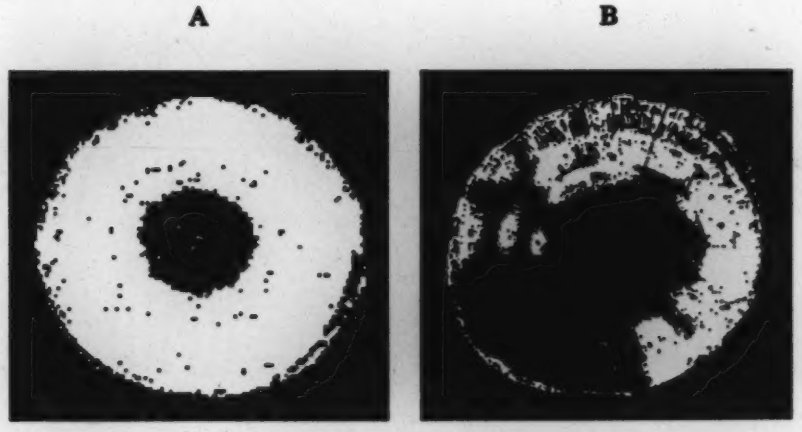
$$K_h = q / (dP/dx) \qquad \text{-equation 2}$$

where q is the flux (volume of water flowing through a unit cross-sectional area per

unit time in m s^{-1}) and dP/dx is the hydraulic gradient (pressure drop per unit length in the direction of flow in Pa m^{-1}). Hydraulic conductivity has units m s^{-1}.

Figure 1(a) shows the actual sapwood disruption caused by the treatments from similarly treated branches of adjacent trees. It can be seen that the desired levels of 40% and 80% sapwood reduction were not achieved. The mean reductions in hydraulic conductivity resulting from the drilling treatments were 45% and 72% respectively (Fig. 1b), and for the following discussion these treatments will be referred to as low disruption and high disruption.

Plate 2. Cross section of a lower branch from a closed canopy tree. (A) Untreated section showing the non-conducting heartwood (centre black) and conducting sapwood (white). (B) The high disruption treated section of the same branch showing the dye stained actively conducting sapwood and non-conducting radial pattern caused by drilling. Plastic pegs were inserted through drill holes to prevent bulk water flow or healing growth.



As holes were drilled through the whole branch, phloem as well as xylem was disrupted. This interference with photosynthate and solute transport in isolation may have caused foliage mortality. The mortality caused by phloem disruption was accounted for in separate treatments. Two other branches within each whorl were

subjected to a low and high level removal of phloem respectively to provide a measure of this mortality. A one centimetre wide band of phloem was peeled from 40% or 80% of the branch over-bark circumference and wrapped with duct-tape to prevent regrowth of cambium during the season. The remaining branch of each whorl served as a control to account for natural foliage mortality over the growing season.

Foliage mortality between crown positions and branch treatments was analysed using an analysis of variance for a nested split-plot design (The SAS Institute).

Physiological responses

Foliage mortality caused by sapwood disruption was further investigated by measuring physiological parameters on ten additional trees at each site. The work was expanded to include open canopy trees as a control for the effects of lower branch age on mortality. Closed canopy trees (where mortality of lower branches may be due to a combination of branch age and low light) were compared with open canopy trees (where branch mortality was presumed to be due only to branch age). Sapwood disruption treatments were applied in the same fashion as for the foliage mortality experiment. The phloem disruption treatments were not used in this experiment as photosynthate disruption was unlikely to have had a significant effect over the short duration of this experiment. Treated trees were left for seven days to allow drought stress to develop in the branches. Conditions were hot and arid during this period. Physiological variables including transpiration and stomatal conductance fluctuate considerably over the course of the day due to varying ambient temperatures and photosynthesis rates. Therefore this diurnal variation was mapped in separate trees, and treated trees were all measured within two hours in late morning during which fluctuations were minimal.

The shade grown trees were also treated 7 days before measurement but since the aim was to measure differences caused by hydraulic effects, not light, equivalent light conditions to open canopy trees were required for all whorls. This was achieved by removing trees from around each experimental tree to allow full sunlight to reach

the lower whorls 20 minutes before taking measurements. Stomatal conductance and transpiration were measured on the terminal 3cm of the main leader of all experimental branches using a steady state porometer (Li-cor Inc. Li-1600). The terminal branch section was then excised and xylem water potential measured immediately using a Scholander pressure bomb (Pms Instrument Co.). Each section was then stored in ice and transported to the lab to measure total leaf area using a flat-bed scanner and image analysis software. Stomatal conductance (g_s) values were corrected for actual leaf areas and porometer boundary layer resistance (R_b = 0.15 s/cm) as follows:

$$g_s = 1 / (R_d + 0.15) [A_t/A] - R_b$$

where R_d = displayed differential resistance (s/cm); A_t = actual foliage area (cm^2), and A = cuvette standard area (cm^2).

Physiological measurements between open and closed canopy trees, upper and lower crowns, and treated branches were analysed using an analysis of variance procedure for a nested split-plot design (The SAS Institute).

As light levels played a major role in this study, measurements were taken using a Decagon ceptometer to quantify the difference in light levels between upper and lower whorls. Readings were taken mid-morning one sunny day in July and again in August. The ceptometer wand was held parallel to the branch to get an average of 10 readings from the bole to the periphery of the crown in the directions of north, south, east and west. Light levels were expressed as a percentage of the light measured in a nearby clearing.

B. Branch xylem hydraulic permeability and anatomy.

Common sampling procedures.

Sixteen additional trees were randomly selected in each of the two stands: 8 open grown with nearly full crowns and 8 exhibiting crown recession. Within each tree, upper and lower whorls were marked corresponding to the whorls used for the foliage mortality and physiology measurements. Within each whorl, one branch was

selected on the basis of having a suitable internode long enough that it could later be divided into three 10cm sections. Each branch was clipped off flush with the stem and immediately placed under water in a cooler. It was then re-cut under water 10 centimeters from the cut end and sealed in iced water within an airtight container. All samples were stored at -15°C. Hacke & Sauter (1996) reported little or no consequence of freezing on hydraulic permeability in *populus* spp.

Hydraulic permeability

Three branch sections were cut underwater from the second internode from the bole end. These sections were approximately 10cm long, the first being at least 20cm from the cut end (two of the sections were used for the treatment calibration for the drilling experiment in section A). This was to ensure that any air that entered the xylem when the initial break was made in the water column during sampling would not be present in the samples. Sections were thawed at room temperature for 24 hours while submerged in filtered (0.2μm) water. Immediately prior to fitting the sample into the permeability measurement apparatus, both ends of each sample were carefully shaved with a razor blade to remove resinous exudate. Exact sample length was measured for the permeability calculations. Each branch was defoliated and total leaf area measured by scanning all leaves with a flat bed scanner coupled to image analysis software.

<u>The permeability measurement apparatus.</u>

The apparatus used a system of hanging water columns (after Booker 1984) to generate a 7.5kPa tension head across the branch section. Tension was used as opposed to pressure, as water columns in tracheids are under tension, therefore one might surmise that this is a more realistic approach than using a pressure potential. A previous study with this system showed a difference in flow rates with the two systems (Silins, U. and Protz, C., unpublished data). This is important as most of the studies conducted in hydraulic architecture to date have used pressure systems (including Mencuccini & Grace 1996), which may not represent natural conditions in stems.

Flow rate measurements.

Samples were perfused in their natural direction of flow with filtered (0.2 μm), degassed water. Water was run for 60 minutes without anti-oxidants, as reports in the literature show that there is no appreciable microbial build up within the tracheids during this length of time (Booker, 1984; Sperry & Tyree, 1988a). Outflow measurements were automated using a computer and scale and outflow temperature was recorded to correct for variations in water viscosity. The flow rate through branch samples was allowed to stabilize for 15 minutes before measurements commenced. After collecting one hour of consistent flow rate data, the perfusate was switched to filtered (0.2μm) degassed acid fuschin dye until a consistent standard colour of outflow was observed. The purpose of the dye was to stain the actively conducting sapwood so that hydraulic permeability could be calculated on a sapwood specific cross-sectional area basis. Dyed samples were frozen to temporarily fix the dye. They were then sectioned and one newly exposed surface was shaved with a razor blade before being scanned. Using Idrisi GIS software, the stained area could be calculated separately from unstained sapwood and heartwood.

Within sapwood, the ease with which the individual 'pipes' or elements conduct water to the foliage was measured as hydraulic permeability. Confusion exists in the literature between the terms hydraulic conductivity (a property of both the viscosity of the fluid and the ability of the porous medium to allow movement of the fluid), hydraulic resistance (the reciprocal of conductivity) and hydraulic permeability (solely a property of the porous medium, as the viscosity of the fluid is already accounted for). Many workers mistakenly refer to hydraulic conductivity, rather than permeability. Hydraulic conductivity was used in calibrating sapwood disruption treatments in part A because it is independent of cross-sectional area. This part of the study will refer to hydraulic permeability on a sapwood area and leaf area basis. The hydraulic permeability (in m^2) expressed per unit area of conducting sapwood (k_S) and per unit area of leaf tissue supported (k_L) were calculated from the following equations;

$$k_S = (q_s * \eta * L) / (dP) \quad \text{-equation 3}$$

$$k_L = (q_L * \eta * L) / (dP) \quad \text{-equation 4}$$

where q_S is the flux ($m\ s^{-1}$) on a sapwood area basis; q_L is the flux ($m\ s^{-1}$) on a leaf area basis; η is the dynamic viscosity of water ($Pa\ s^{-1}$); L is the sample length (m); dP is the 7500 Pa tension difference across the sample. To clarify any differences in results between k_S and k_L, the Huber value - the ratio of sapwood cross-sectional area / total branch leaf area - was calculated for each branch (Tyree & Ewers 1991).

Hydraulic permeability and Huber values were analysed using an analysis of variance procedure for a nested split-plot design (The SAS Institute).

Xylem anatomy

The frozen dye-stained control samples of upper and lower branches used for the conductivity measurements (n = 21) were used to prepare thin cross - sections for microscope slides. The acid fuschin dye used to delineate active sapwood concentrated on the cell walls of the earlywood tracheids. These dye-flow patterns showed that earlywood was responsible for virtually all of the water transport. The slides were analyzed using a digital image processing system consisting of a Sony 3CCD-DXC-930 camera and a Leitz-Wetzlar Dialux 20 EB microscope in conjunction with Northern Lights image analysis software. For each slide (branch) 40 tracheid lumen diameters were measured randomly across the earlywood from the current and penultimate years of growth. Earlywood and latewood widths for each year of growth were measured at a lower magnification.

In order to investigate tracheid lengths and bordered pit frequencies, longitudinal slivers ≥ 1 cm in length were prepared from the earlywood of the current year of growth from each branch sample. Slivers were macerated using the technique described by Pothier & Margolis (1989b). This technique involved submerging the slivers in a solution of 20% v/v nitric acid and 20% v/v chromic acid (1:1) for 14 hours. They were then washed with 1:1 70% ethanol : glycerin and stained with safranin dye to highlight bordered pits. Longitudinal slides were analyzed with the

same image processing system as the cross-sectional slides, and tracheid lengths measured for a random 40 cells per slide (n = 21). Total numbers of bordered pits were counted on both tangential and radial walls for 15 out of the 40 tracheids, selected at random.

Differences in tracheid diameters, lengths, and pit frequencies between upper and lower branches and open and closed canopy trees were analysed using a nested split-plot analysis of variance (The SAS Institute). A similar approach was used for annual ring widths and earlywood ratios, but separate tests were performed for the first 5 years of data and the most recent 5 years.

RESULTS

A. Foliage mortality and physiological responses to imposed drought stress.

In closed canopy trees photosynthetically active radiation (PAR) in mols cm^{-2} s^{-1} was approximately 14% of ambient light for shaded lower crowns, yet nearly full ambient light for upper branches. Open canopy trees by definition experienced nearly full ambient light conditions at both crown positions.

Sapwood disruption increased foliage mortality (Fig. 2a); within whorls at both crown positions, needle mortality on branches that were drilled or girdled was 2-3 times higher than in controls ($p = 0.001$). Sapwood and phloem disrupted (drilled) branches showed greater needle mortality than phloem disrupted (girdled) branches ($p = 0.029$). In all branch treatments dead fascicles were randomly and evenly distributed along branches, often with just one dead needle per fascicle. Natural mortality of lower whorls (including controls) was approximately double the foliage mortality of the upper whorls ($p < 0.001$, $n = 20$). These results show that the lower branches had greater natural foliage mortality and were more susceptible to artificially intensified drought stress. In contrast, xylem disruption in the upper branches had little effect on foliage mortality.

Physiological responses.

These results indicate that these lower branches are much more sensitive to intensified water restriction. Transpiration rates (E) were closely associated with stomatal conductance (g_s) for all branches measured. This indicated that the effect of non-stomatal limitations of transpiration was small between treatments, crown positions, and canopy densities. Thus, results for E are represented only as probability values in the appendices (Table 1.3). Stomatal conductance (Fig.3) was similar in open and closed canopy trees when averaged across control and sapwood disrupted branches from both crown positions ($p = 0.283$). Upper branches had higher g_s than lower branches when averaged across all treatments ($p < 0.001$). Sapwood disrupted branches had lower g_s than control branches in both crown positions in both stand densities ($p < 0.001$). In closed canopy trees, there was a large difference between upper and lower branches ($p = 0.004$), whereas both crown positions were very similar in open canopy trees. This shows that shaded lower branches are experiencing a greater degree of stomatal closure to regulate water loss, while in open canopy trees upper and lower whorls have a similar water supply status. Sapwood disruption treatments had a stronger effect in reducing g_s in closed canopy trees than in open canopy trees ($p < 0.001$). On average, sapwood disruption treatments had similar effects on g_s in both crown positions in open and closed canopy trees because of the strong interaction between treatment and stand density ($p = 0.001$).

Differences in g_s and E responses to sapwood disruption between crown positions and stand densities did not have associated differences in branch xylem water potential (Ψ, Fig. 3). There was no difference in Ψ between open canopy and closed canopy trees ($p = 0.858$), nor between upper and lower crown positions ($p = 0.575$), nor between sapwood disruption treatments ($p = 0.789$). The interaction terms all had Pr > f greater than 0.1. These results indicate that branches regulate xylem water potential successfully during water stress by reducing stomatal conductance to control evaporational water losses.

B. Branch xylem hydraulic permeability and anatomy.

Hydraulic permeability.

Branch hydraulic permeability expressed per unit leaf area (k_L) varied among different crown positions and among trees grown at different densities (Fig. 4a). For both upper and lower branches, k_L was marginally lower in closed canopy trees than in open canopy trees ($p = 0.062$). Averaged across closed and open canopy trees, lower branches had lower k_L than upper branches ($p = 0.033$). There was a marginally significant interaction ($p = 0.070$) between crown position and stand density. In open canopy trees there was negligable difference between upper and lower branches, but in closed canopy trees lower branches had much lower hydraulic permeability.

When expressed on a per unit sapwood area basis, hydraulic permeability (k_S) gave very similar results to k_L (Fig. 4b). Values for k_S were greater in magnitude than for k_L since permeability was divided by a much smaller area.

Huber values are a ratio of sapwood cross sectional area / leaf area subtended (Fig. 4c). Both crown positions had similar sapwood areas supporting a unit leaf area in open canopy and closed canopy trees ($p = 0.354$).

Xylem anatomy.

Mean tracheid lumen diameters (i.e., excluding cell walls) were approximately 15μm (± 0.5μm or 3%, n = 21 trees). Averaged across open and closed canopy trees, upper branches had larger lumen diameters than lower branches ($p = 0.0341$). There was a greater difference between crown positions in closed canopy trees ($p < 0.001$) than in open canopy trees ($p = 0.015$).

Tracheid length and frequency of bordered pits did not differ overall between open and closed canopy trees ($p = 0.437$; 0.858 respectively, Fig. 5b, n = 20 trees), and varied little overall between crown positions ($p = 0.840$; 0.219 respectively). All interactions had Pr >f greater than 0.1. Within the open canopy trees however, upper branches had a lower pit frequency than lower branches ($p < 0.001$) whereas frequencies in both crown positions in closed canopy trees showed little variation ($p = 0.261$).

In addition to variation in xylem anatomy due to light conditions during growth, the proportion of earlywood in annual growth rings declined as light levels fell over time. For lower crown branches earlywood / latewood ratios were similar in open canopy and closed canopy trees for the first 5 years of growth ($p = 0.148$), after which this ratio in open canopy branches remained almost constant while closed canopy lower branches grew progressively less earlywood each year (Fig. 6a, $p = 0.063$). Total annual ring widths for lower crown branches of open canopy and shade grown trees are shown in figure 6 (b). Closed canopy lower branches had marginally greater radial growth in the first 5 years ($p = 0.087$) but growth in both open and closed canopy lower branches declined at a similar rate in the last 5 years ($p = 0.640$). The decline in growth was significant for successive years over this period ($p < 0.001$). Upper branches from open and closed canopy trees only had three complete growth rings which showed no trends in radial growth or earlywood / latewood ratios.

Figure 1. Degree of sapwood disruption in the imposed drought stress experiment, in upper and lower branches (n=18 trees). (a) Conducting sapwood area disruption (% of control + SE). (b) Hydraulic conductivity K_h ($ms^{-1} x10^{-4}$ + SE); low disruption treatments reduced k_h by 45% and high disruption by 72% (p = 0.011).

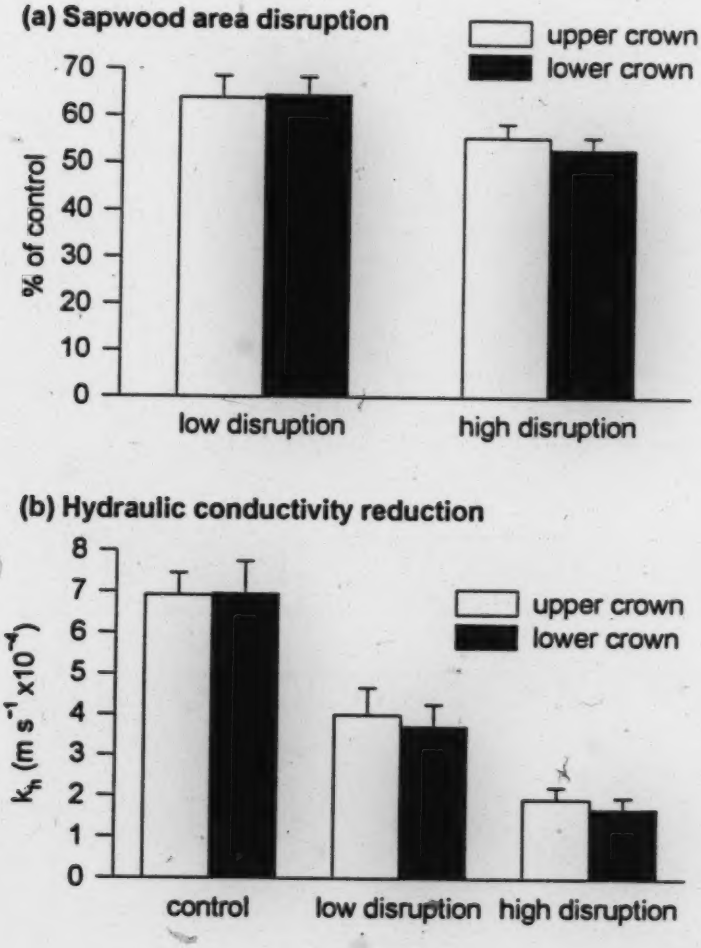


Figure 2. Foliage mortality (% + SE) from low and high sapwood (s/w) disruption treatments, phloem disruption treatments, and controls in upper and lower branches of closed canopy trees (n = 20). Phloem disruption caused greater mortality than controls ($p = 0.0012$) but less than sapwood disruption ($p = 0.029$). Foliage mortality differed between high and low sapwood disruption treatments ($p = 0.005$), and between crown positions ($p < 0.001$).

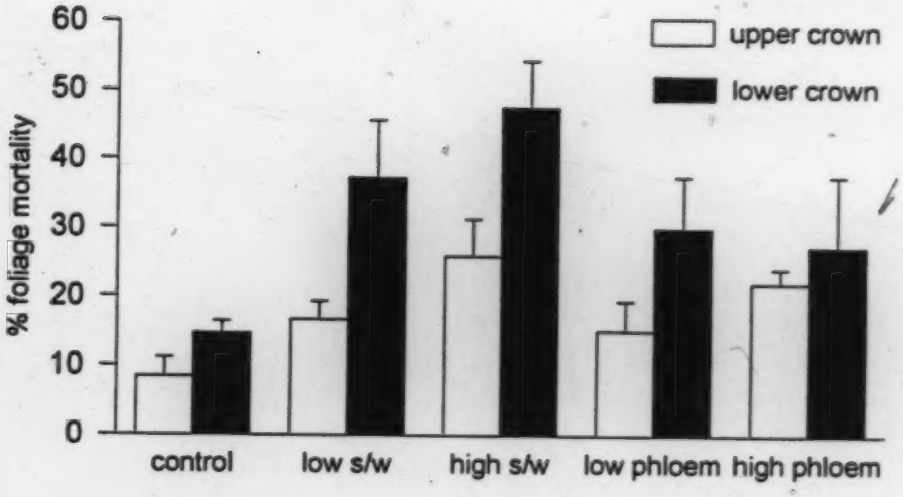


Figure 3. Physiological responses (+ SE) to xylem disruption in upper and lower branches of open and closed canopy trees (n = 20). Stomatal conductance (g_s) and transpiration rates showed identical trends and only g_s (mmol m^{-2} s^{-1}) is shown. Branch water potential (Ψ, MPa) is plotted with corresponding g_s for control and sapwood disrupted branches in (a) closed canopy and (b) open canopy trees. Ψ did not differ significantly with crown position, treatment or tree canopy type. Stomatal conductance differed with crown position ($p < 0.001$) and treatment ($p < 0.001$).

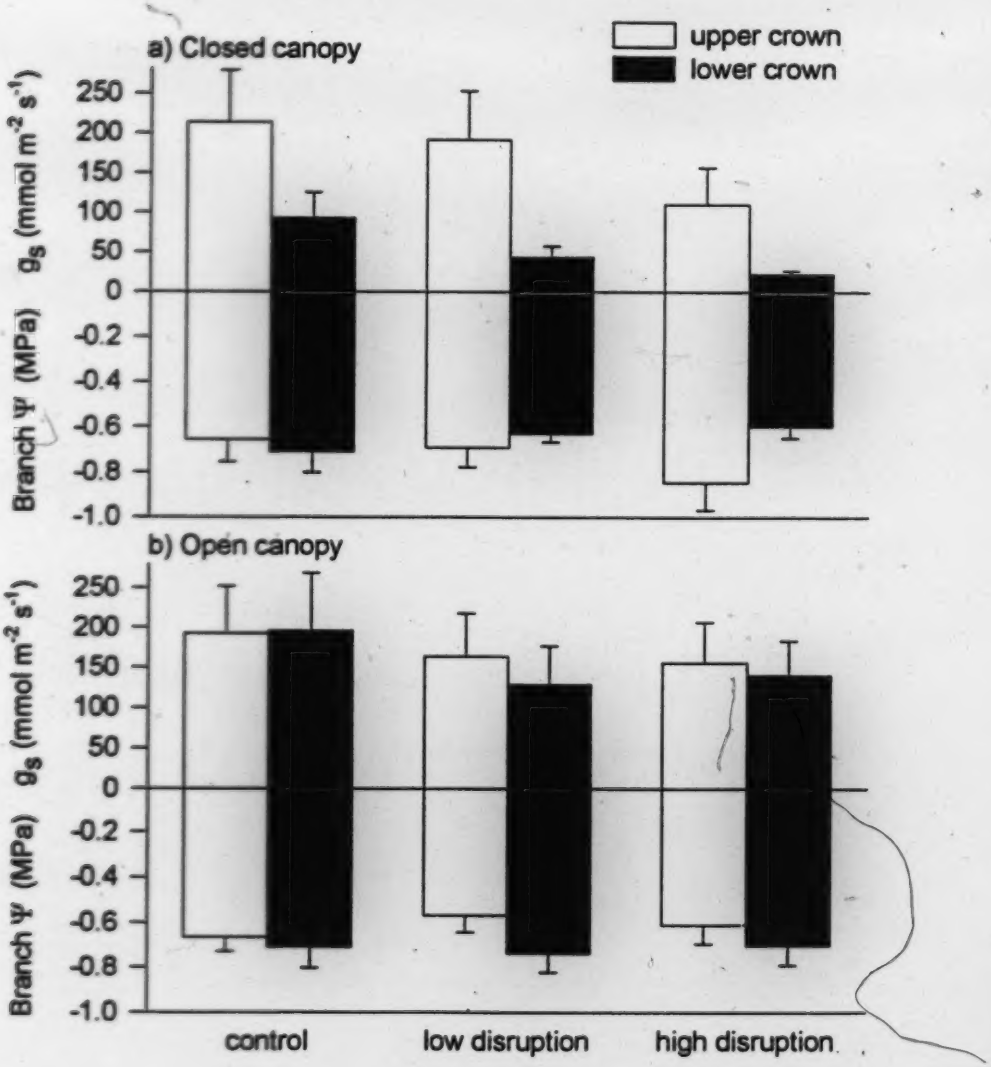


Figure 4. (a) Hydaulic permeability per unit leaf area k_L (m^2 x10^{-5} + SE), (b) Hydraulic permeability per unit sapwood cross-sectional area k_S (m^2 + SE), and (c) Huber values (HV, m^2 m^{-2} + SE) of upper and lower control branches in closed and open canopy trees (n = 24). Both k_L and k_S differed between crown positions (p = 0.033 and 0.052 respectively) and tree canopy types (p = 0.062 and 0.039 respectively).

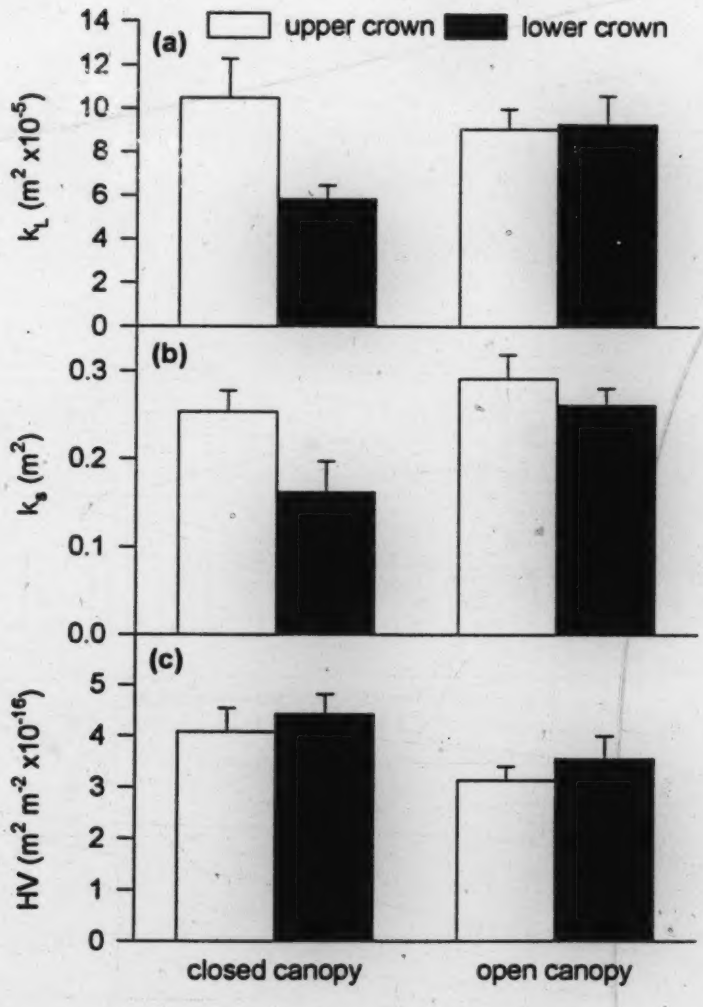


Figure 5. Earlywood xylem tracheid anatomy in upper and lower crown branches of closed and open canopy trees. (a) Tracheid diameters (μm + SE) differed between crown positions ($p = 0.0341$, $n = 21$ trees), but not between stand densities ($p = 0.376$). (b) Tracheid length (μm + SE, $n = 20$ trees) showed little variation between crown positions and stand densities ($p = 0.437$ and 0.840 respectively). (c) The frequency of bordered pits (per 100 μm^{-1} of tracheid length + SE, $n = 20$ trees) differed little between upper and lower branches in closed canopy trees ($p = 0.261$) but the frequency was higher in lower branches of open canopy trees ($p < 0.001$).

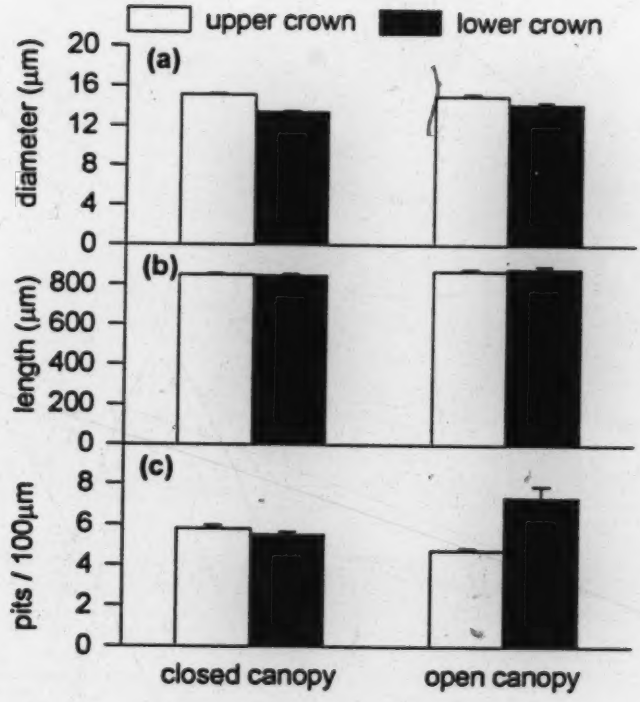
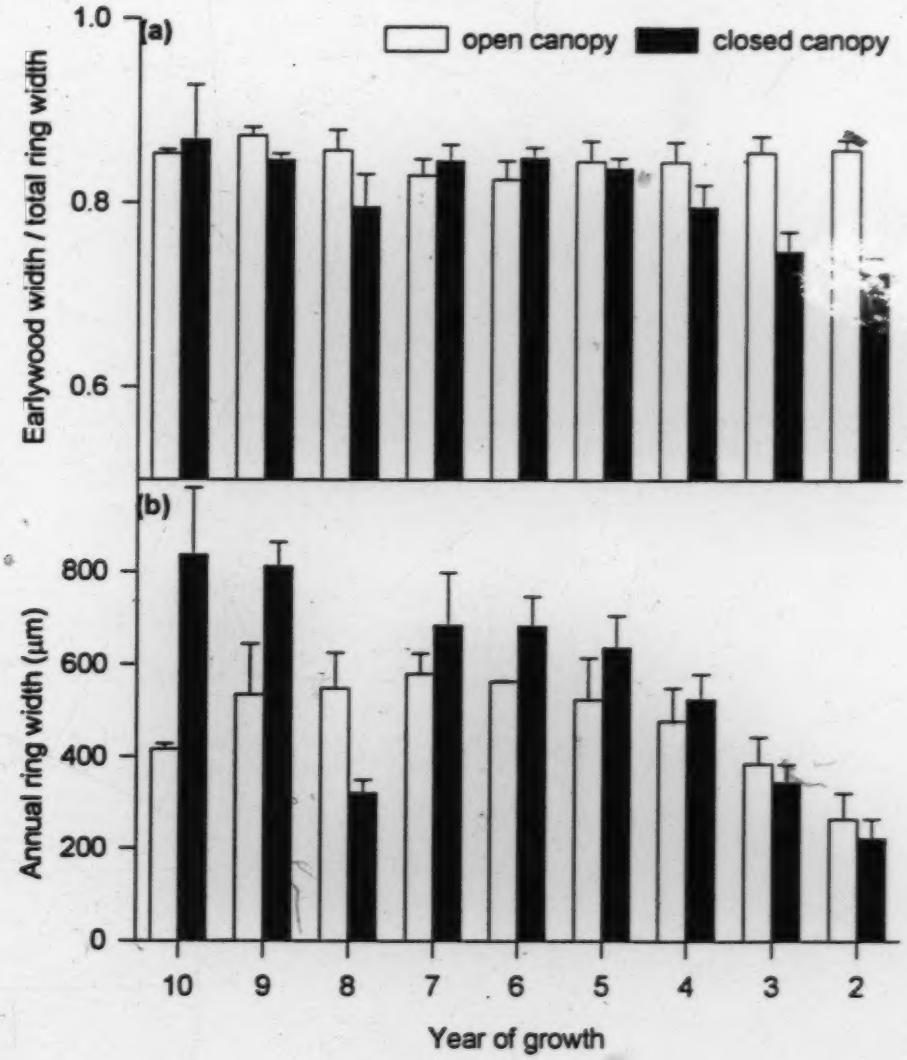


Figure 6. Chronological sequence of annual growth ring measurements in lower crown branches from closed and open canopy trees from the last 10 years (branches were 11 years old). Year 10 corresponds to 1987 growth and year 2 to 1997 growth. (a) Proportion of each years' growth ring width laid down as earlywood (+ SE, n = 20 trees). The earlywood proportion in both canopy densities was similar over the first 5 years ($p = 0.148$) but closed canopy proportions were marginally less in the last 5 years ($p = 0.063$). (b) Total annual growth ring width including both early and latewood (+SE, n = 20 trees). Growth rings were similar overall in both crown densities in the first 5 years ($p = 0.087$) and in the last 5 years ($p = 0.640$).



DISCUSSION

The overall findings validate the hypothesis that crown recession involves more than simply light limitation. The greater foliage mortality in shaded lower crown positions and branches with xylem disruption treatments supports the hypothesis that lower branches experience greater drought stress than upper branches. This is further supported by observations of physiological responses to sapwood disruption. Shade-grown lower crown branches showed greater stomatal closure, presumably to reduce water loss in response to xylem damage than upper branches in the same trees. This is further evidence that lower branches are pre-disposed to drought stress. The hydraulic permeability (k_L) results support the hypothesis that in shade grown (closed canopy) trees the lower whorls are at a competitive disadvantage for water supply compared with the upper crown whorls. The smaller tracheid diameters in shade grown branches support the hypotheses that they have xylem that is less efficient for water transport and that k_S is correspondingly lower in closed canopy lower branches than in upper branches.

The greater foliage mortality resulting from sapwood disruption, in addition to the lower k_L in lower shaded branches are strong indicators that water supply to these crown base branches is limiting. Once xylem growth is influenced by shaded growing conditions, several changes occur in the xylem which reduce the hydraulic permeability. The tracheids produced in the earlywood within each year's growth develop smaller diameter lumens. This results in a reduction in water flow rate theoretically proportional to the fourth power of the lumen diameter (from equation 1) because the resistance to water flow increases due to the greater viscous drag of the tracheid wall.

The investigation of the proportion of earlywood within annual growth rings confirms that growth patterns change in response to declining light levels. While earlywood constituted a constant fraction of the annual growth over 10 years in lower branches of open canopy trees, it declined in the last 5 years in closed canopy lower branches. The last 5 years correspond to complete canopy closure in the stand and a decline in light penetrating to the lower canopy. The consistency of the earlywood

proportion and total annual growth in open canopy lower branches over the last 5 years is a further indication that shade related morphological changes occur independently of age. However, the results suggest that the proportion of earlywood, not total ring width, is most affected by shade. Since the majority of water transport in sapwood occurs through the earlywood (Pothier *et al.* 1989), earlywood : latewood ratios are a major factor associated with the reduced permeability found in lower branches of closed canopy trees.

As the quantity and permeability of xylem declines over successive years of shaded conditions, these lower branches are increasingly pre-disposed to drought stress. Other authors have also found that low light growth conditions can be detrimental to the hydraulic permeability of xylem. Sellin (1993) found stem xylem permeability in *Picea abies* to be 1.4-3.1 times higher in an open-canopy tree than in a closed canopy tree, while Schultz & Matthews (1993) found that xylem tracheid growth can be altered by low light conditions in the stemwood of grapevines. The hydraulic permeability measured in the present study is consistent with the equivalent values of hydraulic conductivity per unit leaf area reported by Sellin (1991) in stem wood of *Picea abies* and by Pothier *et al.* (1989b) in *Pinus banksiana* stemwood. Tracheid dimensions are also consistent with the literature (Pothier *et al.*, 1989b).

After successive years of shaded growth and declining xylem permeability, the water stress increases in lower branches of closed canopy trees. This is illustrated by the sapwood disruption experiment where exacerbating the water stress caused significantly greater foliage mortality in shaded lower branches than in open canopy or upper branches. As the branch water stress increases, the foliage exhibits physiological adaptations to the resulting water shortage. The shaded lower branches in this study showed a significant reduction in stomatal conductance and transpiration rates (compared to upper branches), reflecting the closure of the stomates to conserve water during the day. This enforced 'shutdown' of transpiration reduces the productivity of the foliage, which in turn leads to poorer growth of new xylem for the next season. This process constitutes a feedback cycle, whereby the branch is essentially cutting off its own water supply over time. This supports the notion that

these branches do not have the ability to cope with further restrictions on water supply.

The uniformity of branch-end water potentials (Ψ) between crown positions reflects stomatal control of Ψ in both upper and lower branches. When water stress within the branch is severe, stomatal closure prevents Ψ falling to levels causing catastrophic xylem embolism as described by Jones & Sutherland (1991), and Sperry & Tyree (1988). These findings support the work of Sperry & Pockman (1993) who found leaf water potentials were maintained even when transpiration and stomatal conductance declined dramatically after notching stems of *Betula occidentalis*. This stomatal regulation process also maintains Ψ in upper branches, but probably a smaller reduction in transpirational demand is needed to maintain Ψ than in lower branches. This lower sensitivity to Ψ in lower branches could be because the embolism threshold Ψ is higher in the shade-grown sapwood due to the smaller tracheid diameters being less vulnerable to cavitation (Sellin 1991).

When stomata close during periods of water stress to protect the branch against the formation of embolisms, the result is a shut down of photosynthesis and therefore carbon production. Now the foliage will have insufficient sugars to meet the carbon cost of respiration and would therefore be expected to be a sink, resulting in a carbon concentration gradient to the lower branches (Mäkelä 1995). Crown base branches are also carbon sinks because after successive years of declining water supply and productivity, they have a high ratio of supportive tissue mass (which consumes carbon through maintenance respiration) to photosynthetic tissue mass. Once the consumption : production ratio is greater than one, the branch becomes a carbon drain on the tree, since it is not exporting carbon yet it is still actively respiring. One might speculate that this is exaggerated in trees where the lower branches continued to grow horizontally towards adjacent patches of light from gaps in the canopy, and therefore have a large mass of supportive wood associated with mechanical bracing near the connection with the stem. Such cases might be the first to self-prune as their negative carbon balance is the most severe.

While shade grown lower branches are a carbon sink, it is evident from the

considerable foliage mortality which resulted from the phloem disruption (girdling) treatments that carbon transport is involved in self-pruning. This agrees with the work of Mäkelä (1995) and Zimmermann (1984). The foliage mortality resulting from the phloem disruption treatments far exceeded that of the controls in lower branches. This may reflect a reduced import of sugars from other parts of the tree because the capacity of the phloem is insufficient to nourish the branches, despite the concentration gradient in their favour. The foliage mortality resulting from the phloem disruption treatments in the upper crown also exceeded the controls. The reason is unclear, although it is likely that the disruption of the supply of essential micronutrients and growth hormones to the newly emerged and actively photosynthesizing foliage plays a role.

If the capacity of the phloem of shaded lower branches is insufficient to meet the carbon demands of the foliage, one might speculate that the amount and quality of phloem grown each year declines with low light in the same way as xylem. A study on cucumber seedlings is currently the only one I am aware of to link phloem differentiation with light intensity (Aloni *et al.* 1986), however this may occur in coniferous species. The exact mechanism would probably be a restriction on vascular differentiation in the cambium as a result of a change in auxin concentrations in the branch (Aloni 1987). If this is the case, the carbon supply to the branch is being cut off to prevent it becoming a carbon drain on the tree.

In summary, the lower branches of closed canopy trees are more physiologically responsive to imposed sapwood disruption and have poorer xylem and water conductive properties than equivalent branches in open canopy trees. This suggests that branches at the base of the live crown are experiencing greater water stress than upper branches and are unable to cope with further restrictions to water supply. In contrast to the traditional understanding of a purely light driven mechanism for self-pruning, the process now seems likely to be driven initially by shaded growth conditions causing poor xylem development, leading to water stress and poor carbon relations. This new evidence suggests that crowns do not recede solely due to low light, but that the hydraulic architecture of a branch plays a

significant role in determining its lifespan.

LITERATURE CITED

Aloni, R., Raviv, A., Wolf, A. 1986. Effect of light intensity on the differentiation of phloem anastomoses in cucumber seedlings. Am. J. Bot. 73:618.

Aloni, R. 1987. Differentiation of vascular tissues. Ann. Rev. Plant Physiol. **38**:179-204.

Booker, R.E. 1984. Dye-flow apparatus to measure the variation in axial xylem permeability over a stem cross-section. Plant, Cell and Environment. 7: 623-628.

Edwards, W.R.N., and Jarvis, P.G. 1982. Relations between water content, potential and permeability in stems of conifers. Plant, Cell and Environment, **5**: 271-277.

Jones, H.G., and Sutherland, R.A. 1991. Stomatal control of xylem embolism. Plant, Cell and Environment, **14**: 607-612.

Hacke, A.U., Sauter, J.J. 1996. Drought-induced xylem dysfunction in petioles, branches, and roots of Populus balsamifera L. and Alnus glutinosa (L.) Gaertn. Plant-physiol. **111 (2)** : 413-417.

Maguire, D.A., and Hann, D.W. 1990. Constructing models for direct prediction of 5-year crown recession in southwestern Oregon Douglas-fir. Can. J. For. Res. **20**:1044-1052.

Mäkelä, A. 1995. A carbon balance model of growth and self-pruning in trees based on structural relationships. Forest Science, **43**(1): 7-24.

Mencuccini, M., and Grace, J. 1996. Developmental patterns of above-ground hydraulic conductance in a Scots pine (*Pinus sylvestris*) age sequence. Plant, Cell and Environment, **19**: 939-948.

Natural Resources Canada. 1996. Field guide to ecosites of West-central Alberta. UBC Press.

Pothier, D., Margolis, H.A., and Waring, J. 1989. Patterns of change of saturated sapwood permeability and sapwood conductance with stand development. Can. J. For. Res. **19**: 432-439.

Pothier, D., Margolis, H.A., Poliquin, J., and Waring, R. 1989. Relation between the

permeability and the anatomy of jack pine sapwood with stand development. Can. J. For. Res. **19**: 1564-1570.

Ryan, M.G., Yoder, B.J. 1997. Hydraulic limits to tree height and tree growth. Bioscience **47(4)**:235-242.

Sampson, D.A., and Smith, F.W. 1993. Influence on canopy architecture on light penetration in lodgepole pine (*Pinus contorta* var. *Latifolia*) forests. Agricultural and Forest Meteorology, **64**:63-79.

Schultz, H.R., and Matthews, M.A., 1993. Xylem development and hydraulic conductance in sun and shade shoots of grapevine (*Vitis vinifera* L.): evidence that low light uncouples water transport capacity from leaf area. Planta, **190**:393-406.

Sellin, A. 1991. Hydraulic conductivity of xylem depending on water saturation level in Norway spruce (*Picea abies* (L.) Karst.). Plant Physiol., **138**: 466-469.

Sellin, A. 1993. Resistance to water flow in xylem of *Picea abies* (L.) Karst. trees grown under contrasting light conditions. Trees, **7**: 220-226.

Sperry, J.S., Donnelly, J.R., and Tyree, M.T. 1988. A method for measuring hydraulic conductivity and embolism in xylem. Plant, Cell and Environment. **11**:35-40.

Sperry, J.S., Pockman, W.T. 1993. Limitation of transpiration by hydraulic conductance and xylem cavitation in *Betula occidentalis*. Plant Cell and Environment **16**:279-287.

Sperry, J.S., Saliendra, N.Z., Pockman, W.T., Cochard, H., Cruiziat, P., Davis, D.,

Sperry, J.S., and Tyree, M.T. 1988. Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress ? Answers from a model. Plant Physiol., **88**: 0574-0580.

Sperry, J.S., and Tyree, M.T., 1990. Water-stress-induced xylem embolism in three species of conifers. Plant, Cell and Environment. **13**: 427-436.

Tyree, M.T., and Ewers, F.W. 1991. Tansley Review No. 34. The hydraulic architecture of trees and other woody plants. New Phytol., **19**: 345-360.

Waring, R.H., Newmann, K., and Bell, J. 1981. Efficiency of tree crowns and

stemwood production at different canopy leaf densities. Forestry, **54**:129-137

Waring, R.H., Pitman, G.B. 1985. Modifying lodgepole pine stands to change susceptability to mountain pine beetle attack. Ecology **66**: 889-897.

Yang, S., and Tyree, M.T. 1994. Hydraulic architecture of *Acer saccharum and A. rubrum*: comparison of branches to whole trees and the contribution of leaves to hydraulic resistance. Journal of Experimental Botany, **45**: 179-186.

Zimmermann, M. 1978. Hydraulic architecture of some diffuse porous trees. Can. J. Bot., **56**: 2286-2295.

Zimmermann, M.H. 1983. Xylem structure and the ascent of sap. Springer, Berlin, Heidleburg, New York.

Chapter 3.

Synthesis

SYNTHESIS

The role of crown recession in a broader context is to maximize efficiency of carbon allocation within the growing tree, which is fundamental to questions of crown dynamics and tree growth. It is useful to consider why it is that coniferous species exhibit apical dominance and why they invest the majority of available resources in height growth. Gymnosperms are believed to be primitive to angiosperms in an evolutionary sense, and their reproductive ecology supports this. Most conifer species are wind pollinated, and their apically dominant canopy form may have been selected by evolutionary pressures that favour reaching a sufficient height to maximize long-distance seed dispersal. The seed cones of an individual tree that grows higher than the surrounding canopy (dominants) should have the greatest access to air currents, allowing the seed to be dispersed over a greater distance and thus find suitable seedbeds more often. Such a tree would therefore have a greater representation of its genotype in the next generation. From the perspective of crown recession, an efficient dominant tree has no use for lower branches that are neither photosynthetically productive nor able to produce seed that can be successfully dispersed. It is not surprising that a mechanism has evolved to eliminate these largely non-functional branches.

This argument also explains why trees of the same species reach different maximum heights in different situations - a question that been much studied (Ryan & Yoder 1997). If conifers only have one function - to reproduce - there is no reason for them to grow taller than necessary to successfully disperse seed. For an open canopy tree with a full live crown, seed will disperse from cones on any of the branches quite effectively, as they all have access to air currents and wind. In this case there would be little efficiency gain from self-pruning as lower branches can contribute to reproductive success. In high elevation stands, canopy height is often much less for a given conifer species than in low lying areas (Ryan & Yoder 1997). This could be attributable to site qualities or wind burn of emergent buds, but such trees are probably exposed to very frequent winds and thermal air currents that could effectively disperse seed over a long distance regardless of tree height. Therefore

there is no reason for a tree to invest valuable limited resources in height growth when it could be utilized for seed production.

This research has economic implications to the forest industry. The last decade has seen an increasing awareness among the forest sector in western Canada that the forest resource is capable of being depleted unless it is managed more responsibly and intensively. This realization, as well as the generally slow growth rates of conifers in Alberta, has provoked the adoption of more intensive silvicultural management as has long been practiced throughout Europe. Among the most important practices are partial cut systems and commercial thinning. As both of these are relatively new practices in western Canada, it is vital to know how boreal species and ecosystems will be affected by partial cutting over the course of whole rotations. This research will play a role in deciding optimal residual densities after partial cutting and thinning. A crown recession factor can be incorporated into stand growth models to predict future yields and ultimately refine the crucial calculations of sustainable annual allowable cuts. Individual stand prescriptions could incorporate predicted rates of crown recession for different site conditions and species mixtures. The optimal time in the rotation to thin and the target residual density that would yield the greatest production efficiency per hectare over the rotation could then be calculated. On an individual tree basis, this information could be used to manage stand density to maintain the optimal live crown ratio for growth and vigour of the trees by pre-commercial and commercial thinning at different times over the course of stand development.

Optimizing live crown ratio also has applications to the practice of pruning in high value (sawlog or veneer quality) stands such as white pine and Douglas-fir in the British Columbia interior. Silviculturalists need to predict the desired live crown ratio for the maintenance of optimal mean annual increment and the shortest growth lag after pruning or thinning. Further work is needed to determine the relationship between stand density and stand age at which light limitation begins to cause hydraulic stagnation of lower crowns. It is also important to understand the relationship between stand density and the rate of crown recession once stagnation

has begun. These relationships would give managers greater insight when scheduling silvicultural operations in order to avoid lower crown stagnation and maintain periodic annual increments and stand productivity.

Further research is necessary to investigate the relationship between light levels, phloem differentiation and phloem loading in the context of self-pruning. This might involve measuring phloem loading in upper and lower branches, in conjunction with stable carbon isotope labeling experiments to determine the extent of carbon movement into lower branches. This could be achieved by enclosing upper branch foliage in polythene bags filled with $^{13}CO_2$ and analyzing sections of stem and branches obtained periodically throughout the crown to measure its import into lower branches. If lower branches are not producing sufficient carbon to meet respiration requirements, and little carbon is being imported, this would suggest phloem capacity is limiting carbon movement.

The light-hydraulic mechanism for branch stagnation and decline presented in this study may also be the mechanism responsible for the stagnation of whole stands after canopy closure in *Pinus contorta* - a phenomenon commonly observed in the western boreal forest. Keane and Weetman (1987) suggested such a mechanism for stand level stagnation. In these dense shaded stands, stems may have low hydraulic permeability due to poor sapwood quality, causing height and volume growth to virtually cease. If the stagnation in these stands is due to low permeability xylem, they might be slow to increase growth rates in response to stand thinning because the existing vasculature would have insufficient capacity to meet the greater water demand of the foliage once it is receiving more light.

The effect of low light on xylem hydraulic permeability may also be a major factor in self-pruning in other coniferous species such as boreal Jack pine. This mechanism may be less important or commence at a much greater stand age in other species with a higher tolerance of shade (lower foliar photosynthetic compensation points), such as spruce or fir, however.

A more complete understanding of the physiological processes of crown dynamics and stand growth are fundamental in predicting stand responses to

silviculture. This research has shed new light on branch hydraulic architecture as a principle factor in the self-pruning process, and perhaps further work will implicate crown hydraulic relations with stand level stagnation.

Literature cited

Keane, M.G., and Weetman, G.F. 1987. Leaf area-sapwood cross-sectional area relationships in repressed stands of lodgepole pine. Can. J. For Res. 17 :205-209.

Ryan, M.G., Yoder, B.J. 1997. Hydraulic limits to tree height and tree growth. Bioscience 47(4):235-242.

Chapter 4.

Appendices: Statistical analysis summary tables

Table 1.1. Results of ANOVA's of (a) hydraulic conductivity reduction, and (b) sapwood area disruption in the imposed drought stress experiment. Upper and lower branches (position) were disrupted by high and low disruption treatments (treatment). Values indicate P>f.

a.

	d.f.	k
site	1	0.008
tree(site)	16	0.759
position	1	0.289
tree*position (site)	17	0.406
treatment	2	0.011
position*treatment	2	0.706
tree*treatment(site)	34	0.686
n (trees)		18

b.

	d.f.	Area
position	1	0.458
treatment	2	<0.001
position*treatment	2	0.951
n (trees)		18

Table 1.2. Results of ANOVA's of foliage mortality due to imposed drought stress in upper and lower branches (position) of closed canopy trees. Values indicate P>f.

	d.f.	
site	1	0.734
tree(site)	18	0.272
position	1	<0.001
site*position	1	0.004
tree*position(site)	18	0.879
treatment	4	0.005
site*treatment	4	0.689
tree*treatment(site)	72	0.233
position*treatment	4	0.361
site*position*treatment	4	0.867
n (trees)		20

Table 1.3. ANOVA results for measurements of physiological responses to xylem disruption in upper and lower branches (position) of open and closed canopy trees (density). P>f values for stomatal conductance (g_s), transpiration rates (E) and branch water potential (Ψ) are shown.

	d.f.	g_s	E	Ψ
density	1	0.283	0.322	0.858
tree(density)	18	<0.001	<0.001	<0.001
position	1	<0.001	0.008	0.575
density*position	1	0.004	0.005	0.281
tree*position(density)	18	0.057	0.002	<0.001
treatment	2	<0.001	0.005	0.789
density*treatment	2	<0.001	0.608	0.504
tree*treatment(density)	36	0.779	0.406	0.058
position*treatment	2	0.305	0.284	0.107
density*position*treatment	2	0.643	0.269	0.021
n (trees)		20	20	20

Table 1.4. Results of ANOVA's of hydraulic permeability per unit leaf area (k_L), hydraulic permeability per unit sapwood cross-sectional area (k_S) and Huber values (HV) in upper and lower control branches in closed and open canopy trees. Values indicate P>f.

	d.f.	k_s	k_L	HV
density	1	0.039	0.062	0.354
tree(density)	22	0.258	0.106	0.023
position	1	0.052	0.033	0.789
position*density	1	0.235	0.070	0.559
n (trees)		24	24	24

Table 1.5. ANOVA results of tracheid diameter, length, and bordered pit frequency in earlywood xylem of upper and lower crown branches (position) of (a) closed and open canopy trees (density), (b) and (c) separately within each density. Values indicate P>f.

a. Full model

	d.f.	Diameter	Length	Pits / 100μm
density	1	0.375	0.437	0.858
tree(density)	18	<0.001	<0.001	<0.001
position	1	0.034	0.840	0.219
density*position	1	0.368	0.826	0.114
tree*position(density)	18	<0.001	<0.001	<0.001
n (trees)		21	20	20

b. Open canopy trees

	d.f.	Diameter	Length	Pits / 100μm
tree	4	<0.001	<0.001	<0.001
position	1	0.015	0.368	<0.001
position*tree	4	<0.001	<0.001	<0.001
n (trees)		5	5	5

c. Closed canopy trees

	d.f.	Diameter	Length	Pits / 100μm
tree	14*	<0.001	<0.001	<0.001
position	1	<0.001	0.969	0.261
position*tree	14*	<0.001	<0.001	<0.001
n (trees)		16	15	15

(* 15 for diameter measurements)

Table 1.6. ANOVA results of a chronological sequence of annual growth ring measurements in lower crown branches from closed and open canopy trees (density) from the last 10 years. The first 5 years (10-6) and the last 5 years (5-1) were analyzed separately. P>f values for the ratio of earlywood width to total ring width for each year, and total growth ring width are shown.

	d.f.	width (10-6)	width (5-2)	ratio (10-6)	ratio (5-2)
density	1	0.087	0.640	0.148	0.063
year	4	0.291	<0.001	0.955	0.189
density*year	4	0.167	0.573	0.053	0.751
n (trees)		48	83	48	83